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Analysis of total phenolic content and antioxidant activity in Turi leaf extract (*Sesbania grandiflora* L.) with the use of different solvents

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ABSTRACT
This study aims to evaluate the effect of solvent types on the total phenolic content and antioxidant activity of turi leaf extract (Sesbania grandiflora). The types of solvents used include ethanol, ethyl acetate, and n-hexane. The total phenolic content was measured using the Folin-Ciocalteu method with gallic acid as a standard, while the antioxidant activity was determined using the DPPH quenching method. The results showed that the highest total phenol content was obtained from ethyl acetate solvent (12.31 µg/mL), followed by ethanol (9 µg/mL) and n-hexane (6.34 µg/mL). However, antioxidant activity based on IC50 showed different results, where the extract with n-hexane solvent had the highest antioxidant activity (IC50 = 142.9 µg/mL) compared to ethyl acetate (IC50 = 245.47 µg/mL) and ethanol (IC50 = 271.8 µg/mL). These differences indicate that the type of solvent affects not only the amount but also the type of phenolic compounds and other bioactive compounds in the extract. This study highlights the importance of selecting an appropriate solvent to enhance the extraction efficiency of phenolic compounds and antioxidant potential.

Keywords:
Phenolic Content and Antioxidant Activity; Solvents; Sesbania grandiflora L.

Introduction

leaves (*Sesbania grandiflora L.*) have long been recognized for their potential as a source of bioactive compounds beneficial to human health (Kuttiappan et al., 2024). One group of compounds found in turi leaves is phenolic, which is known to have significant antioxidant activity (Mussagy et al., 2023). This antioxidant activity is important in protecting body cells from damage caused by free radicals, which can contribute to the development of various degenerative diseases. These leaves are known to contain various bioactive compounds, including flavonoids, alkaloids, and phenolics, which play a crucial role in antioxidant activity (Abubakar et al., 2024; Kale et al., 2012). Antioxidant activity is the primary focus of many studies due to its ability to neutralise free radicals that can cause cell and tissue damage, thereby contributing to the development of various chronic diseases, including cancer and cardiovascular disease (Vo et al., 2023).

The use of solvents in the extraction of bioactive compounds from plants is a crucial factor that can significantly impact the efficiency and yield of the extraction process (Vo et al., 2024). Some common solvents used in plant extraction are ethanol, n-hexane, and ethyl acetate. Each solvent has different solubility for certain types of compounds, thus affecting the specificity and quantity of compounds extracted from turi (Allay et al., 2024). Previous studies have shown that extraction with various solvents can produce significant differences in the phenolic content and antioxidant activity of plant extracts. Ethanol, for example, is known to extract polar compounds such as flavonoids and tannins, which often have high antioxidant activity (Paes et al., 2024). On the other hand, non-polar solvents such as n-hexane tend to be more effective in extracting

lipophilic compounds, while ethyl acetate is in between the two in its affinity for these compounds (Tadege et al., 2024).

The use of n-hexane, ethanol, and ethyl acetate solvents in the extraction of turi leaves aims to compare the effectiveness of each solvent in extracting bioactive compounds that act as antioxidants. Each solvent has a different polarity, enabling the extraction of various types of compounds from turi leaves (Dong et al., 2025). N-hexane, which is non-polar, tends to extract non-polar compounds such as lipids and certain phytochemicals. At the same time, ethanol and ethyl acetate, which are more polar, are more efficient in extracting phenolic and flavonoid compounds that are known to have high antioxidant activity. Comparison of the antioxidant activity of the extracts produced with the three solvents will provide an overview of which solvent is most effective in obtaining compounds with the highest antioxidant activity from turi leaves.

Additionally, the selection of the three solvents was based on the chemical characteristics of phenolic compounds, which are generally polar to semi-polar. Phenolics in turi leaves are known to be composed of various groups, including flavonoids, tannins, and phenolic acids, which have different solubilities depending on the length of the carbon chain and the number of hydroxyl groups they have. Therefore, the use of solvents with varying polarity ranges, n-hexane as a non-polar solvent, ethanol as a polar solvent, and ethyl acetate as a semi-polar solvent, is expected to be able to extract specific phenolic groups according to their polarity properties, so that it can be determined which solvent is most effective in producing extracts with the best antioxidant potential.

Methods

Materials and Tools

The materials used in this study are Turi leaf extract, Folin–Ciocalteu Reagent Sigma-Aldrich brand, DPPH· (1,1-*diphenyl-2-picrylhydrazyl*) Sigma-Aldrich brand, Ethanol, ethyl acetate, and n-hexane Merck brand with pro analysis purity level (p.a.), Vitamin C Sigma-Aldrich brand. The tools used in this study were Pyrex brand measuring cups with varying capacities (10 mL, 50 mL, 100 mL), Pyrex brand beakers with capacities of 50 mL to 500 mL, Kimble brand test tubes with a standard size of 15 mL, Eppendorf brand pipettes (droppers and measuring pipettes) for accuracy of liquid measurements, Pyrex brand measuring flasks with capacities of 10 mL, 25 mL, and 50 mL, CoorsTek brand porcelain cups for the evaporation or drying process.

Preparation of plant extracts

Dried turi (*Sesbania grandiflora L.)* leaves were air-dried at room temperature, protected from light, until the moisture content stabilized, then ground into powder. Extraction using a maceration method using three solvents: ethanol, ethyl acetate, and n-hexane, with a sample-to-solvent ratio of 1:10 (100 g powder: 1000 mL solvent). Maceration was carried out for 7 hours at room temperature, a time referenced in previous research that demonstrated that this duration yields optimal phenolic extraction without degradation of bioactive compounds (Shafodino et al., 2024).

After maceration, the mixture is then filtered using Whatman No. 1 filter paper. The filtrate was then evaporated using a rotary evaporator at 40–50°C to obtain a viscous extract. The extract was then dried in a low-temperature drying oven at 40°C to remove any remaining solvent. The resulting crude extract was weighed, and the yield was calculated using the following equation (1).

$$Yield\ (\%) = \frac{Dry\ extract\ weight\ (g)}{Initial\ sample\ weight\ (g)} x100\% \qquad (1)$$

Total phenolic content (TPC) and Characterization using laboratory tests

Total phenol was determined using the Folin–Ciocalteu reagent with gallic acid as a standard. Absorbance was measured using a UV–Vis spectrophotometer at a maximum wavelength of 765 nm after a 30-minute incubation at room temperature in the dark. Phenol

concentration was expressed as gallic acid equivalent (µg GAE/mL) (Zugazua-Ganado et al., 2024).

DPPH TEST

Antioxidant activity was determined using DPPH (1,1-*diphenyl-2-picrylhydrazyl*) free radicals. Extracts were prepared in several concentration ranges (20, 40, 60, 80, and 100 µg/mL). A total of 300 µL of sample was added to 2,700 µL of 60 µM DPPH solution and incubated for 60 minutes at 37°C in the dark. Absorbance was measured at 517 nm. The percentage inhibition was calculated using the equation (2).

$$\%\ Inhibisi = \frac{A_c - A_s}{A_C} x100\% \qquad (2)$$

where Ac is the absorbance of the control and As is the absorbance of the sample. The linear regression curve plotting the extract concentration against the percentage of DPPH radical inhibition determined the IC50 value (Dawa et al., 2021).

Results and Discussions

This study was conducted through a series of laboratory tests to determine the total phenolic content and antioxidant activity of turi leaf extract using three types of solvents: ethanol, ethyl acetate, and n-hexane. All graphs shown in the manuscript, including the gallic acid standard curve and the IC50 determination curve, are the results of this research experiment, not the results of citations or data from previous studies. The regression equation shown is used solely calculate the phenolic content and IC50 value, not as the main method of the study. Therefore, this study is not a mathematical/regression approach, but rather a pure laboratory test.
This study aims to analyze the total phenolic content and antioxidant activity in turi leaf extract (*Sesbania grandiflora*) using different solvents. The solvents used in this study were ethanol, ethyl acetate, and n-hexane. The data obtained include the total phenolic content and antioxidant activity of each extract.

Total Phenolic Content

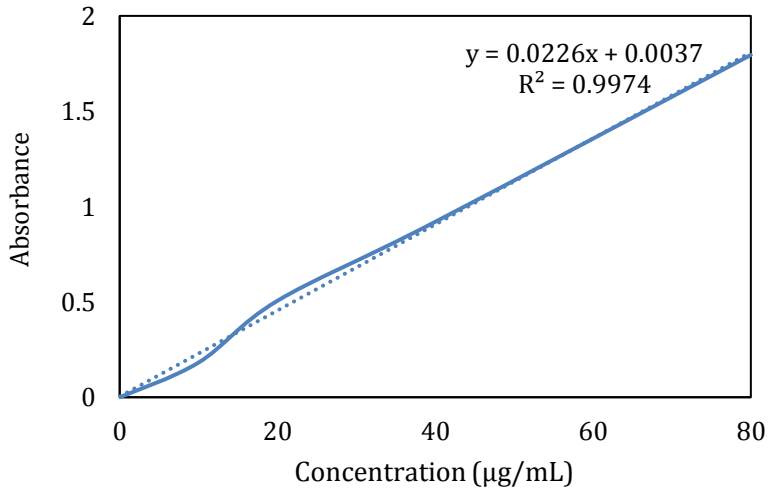

Figure 1. Standard graph of gallic acid

Figure 1 shows total phenolic measurements using the gallic acid standard curve. In the graph, the X-axis represents the standard concentration of gallic acid (µg/mL), while the Y-axis shows the absorbance value. The regression equation in the graph was used to calculate the phenolic concentration in the sample. The results obtained showed that ethyl acetate solvent

produced the highest phenolic content (12.31 µg/mL), followed by ethanol (9 µg/mL), and n-hexane (6.34 µg/mL). This difference is influenced by the polarity of the solvent—ethyl acetate, which has moderate polarity, is able to attract phenolic compounds more optimally than ethanol (polar) or n-hexane (non-polar).

The gallic acid calibration curve was made by measuring the absorbance of various concentrations of standard gallic acid solutions. Figure 1 shows that the absorbance increases linearly with increasing gallic acid concentration, confirming the suitability of this calibration curve for determining total phenolic content in the extracts. The resulting graph shows a linear relationship between gallic acid concentration and absorbance, which is expressed by the linear regression equation (3) is:

$$y=0,0226x+0,0037 \quad (3)$$

where a determination coefficient value of R^2 = 0.9974, this graph shows a robust correlation between the concentration of gallic acid and the measured absorbance. The measurement results show that the turi leaf extract contains a specific concentration of total phenols, as determined by using the regression equation from the gallic acid calibration curve. The determination coefficient value R2, which is close to 1, indicates that the method used is very accurate and reliable for measuring total phenol. so that this regression equation can be used to determine the concentration of total phenol in turi leaf extract.

Different solvents have varying polarities, which affect the ability to extract phenolic compounds from turi leaves (Table 1). Based on the data provided, the highest total phenol content was obtained using ethyl acetate (12.31 µg/ml), followed by ethanol (9 µg/ml), and the lowest was with n-hexane (6.34 µg/ml). This indicates that more polar solvents tend to be more effective in extracting phenolic compounds from turi leaves. Solvent polarity plays a crucial role in the extraction efficiency of phenolic compounds from plant materials, such as turi leaves. Phenolic compounds are organic compounds that have a hydroxyl group (-OH) attached to an aromatic ring. The polarity of these compounds varies, which makes solvent selection a critical factor in the extraction process (Liu et al., 2024).

Table 1. absorbance values of turi leaf extract with various types of solvents

Sample	Absorbance	Average Absorbance	Total Phenol Content y=0,0226x+0,0037
Turi leaf extract with ethanol solvent	0,212 0,211 0,211	0,211	9 µg/ml
Turi leaf extract with ethyl acetate solvent	0,282 0,283 0,282	0,282	12,31 µg/ml
Turi leaf Extract with n-hexane solvent	0,147 0,147 0,148	0,147	6,34 µg/ml

Ethyl acetate has medium polarity, with a dielectric constant of approximately 6. Known for its ability to dissolve polar and non-polar organic compounds, ethyl acetate is often used in the extraction of phenolic compounds because of its balance between polarity and non-polarity (Ogunlakin et al., 2023). Data shows that the use of ethyl acetate produces the highest total phenol content (12.31 µg/ml). Ethyl acetate is capable of dissolving various types of phenolic compounds found in turi leaves, including both polar and non-polar (Sangaraju et al., 2025).

Ethanol has a higher polarity than ethyl acetate, with a dielectric constant value of around 24.5. Ethanol is an effective polar solvent for extracting polar phenolic compounds (Echenique et al., 2024). Although ethanol has a higher polarity than ethyl acetate, the extraction yield of phenol from turi leaves using ethanol is lower (9 µg/ml). This is because some phenolic compounds

present in turi leaves are more easily extracted by solvents with medium polarity, such as ethyl acetate, compared to highly polar solvents such as ethanol.

n-Hexane is a non-polar solvent with a dielectric constant value of around 1.9. As a non-polar solvent, n-hexane is less effective in dissolving polar compounds such as phenol. This is reflected in the data showing that the total phenol content extracted with n-hexane was the lowest (6.34 µg/ml). Because phenolic compounds are generally polar, non-polar solvents such as n-hexane are ineffective in dissolving these compounds, resulting in a low extraction yield. (Alonso-Riaño et al., 2021).

These data indicate that solvents with medium polarity, such as ethyl acetate, are the most effective for extracting phenolic compounds from turi leaves. Solvents that are too polar, such as ethanol or too non-polar, such as n-hexane are not as effective as ethyl acetate. This shows the importance of selecting a solvent that suits the characteristics of the compound to be extracted. The total phenol content in a material, especially plant material, is often closely related to its antioxidant activity. Phenolic compounds, which are a large group of phytochemical compounds, including flavonoids, tannins, and phenolic acids, have a chemical structure that is able to donate hydrogen atoms or electrons and eliminate free radicals, thereby preventing oxidative damage to cells.

Antioxidant Content

The antioxidant activity of turi leaf extract was determined by the DPPH (2,2-*diphenyl-1-picrylhydrazyl*) scavenging method using UV-Vis spectrophotometry. Wavelength Determination The purpose of the maximum wavelength is to determine the wavelength with maximum absorption, namely when the colored compound is formed optimally, thereby achieving the most incredible sensitivity (Anitha et al., 2021).

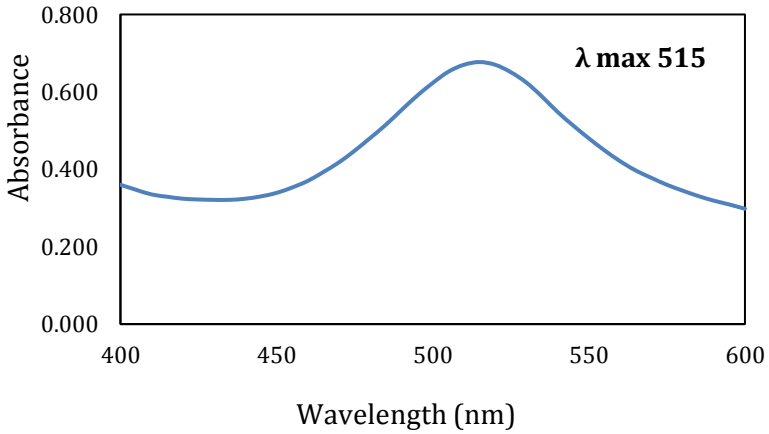

Figure 2. Maximum Wavelength Graph

Antioxidant activity testing takes place experimentally using the DPPH method. Figure 2 displays a graph of the maximum wavelength determination, with the X-axis representing the wavelength (nm) and the Y-axis representing the absorbance value. Meanwhile, the IC50 was determined by graphing the relationship between extract concentration (X-axis) and the percentage inhibition of DPPH radicals (Y-axis). To determine the IC50 value for each extract, one can use the resulting regression equation

The results showed that the n-hexane extract had the lowest IC50 value (142.9 µg/mL), thus possessing the highest antioxidant activity. This finding does not directly align with the total phenolic content, as other non-polar bioactive compounds are also extracted by n-hexane and contribute to antioxidant activity. This demonstrates that antioxidant activity is influenced not only by the amount of phenolics but also by the type of phenolics, the presence of lipophilic

compounds, and the composition of other bioactive compounds, which can contribute to differences in IC50 values even at low phenolic concentrations. Table 2 show the antioxidant Activity of Vitamin C.

Table 2. Absorbance of vitamin C concentration

Concentration	Average absorptance	%Inhibition
10	0,636	6,05613
20	0,62	8,419498
40	0,507	25,11078
80	0,045	93,35303

Table 3. IC50 values of Samples

Sample	Liner Regression Equations	IC50
Turi leaf extract with ethanol solvent	y = 2,25x - 0,4775 R^2 = 0,998	271.8
Turi leaf extract with ethyl acetate solvent	y = 5,125x - 7,2487 R^2 = 0,997	245.47
Turi leaf Extract with n-hexane solvent	y = 8,0769x - 12,397 R^2 = 0,996	142,9

The results showed that vitamin C had an IC50 value of 22.37 µg/ml (Table 3). Previous studies have demonstrated a strong positive correlation between the total phenol content in plant extracts and antioxidant activity, as measured by various methods including DPPH (2,2-*diphenyl-1-picrylhydrazyl*), ABTS (2,2'-azino-bis(3-*ethylbenzothiazoline-6-sulfonic acid*)), and FRAP (*Ferric Reducing Antioxidant Power*). The higher the total phenol content, the greater the observed antioxidant activity. This is due to the ability of phenolic compounds to donate electrons or hydrogen atoms, thereby neutralizing free radicals (Mavric-Scholze et al., 2025).

However, despite the positive correlation, the total phenolic content and antioxidant activity values are not always identical due to several factors. Not all phenolic compounds exhibit the same level of antioxidant activity. For example, certain flavonoids exhibit higher antioxidant activity than specific phenolic acids. In plant extracts, phenolic compounds can interact with other compounds such as vitamin C, carotenoids, and anthocyanins, which also exhibit antioxidant activity. These interactions can increase or decrease the total antioxidant activity (Hu et al., 2025). The method used to extract phenolic compounds can affect the composition and concentration of phenolic compounds in the final extract. Solvents, temperature, and extraction time can affect the results. The parts of the plant used (leaves, fruits, skins, seeds) can have different phenolic contents and antioxidant activities. For example, fruit skins often contain higher phenols than the flesh of the fruit. Processes such as drying, heating, and storage can affect the stability of phenolic compounds and, consequently, antioxidant activity.

The results showed that although the ethyl acetate produced the highest total phenol content, the antioxidant activity measured through IC50 was not linearly aligned with the total phenol content. A lower IC50 indicates higher antioxidant activity. In this case, the extract with n-hexane showed the highest antioxidant activity (lowest IC50) despite having the lowest total phenol content. The type of phenolic compounds extracted by n-hexane has more potent antioxidant activity compared to those extracted by ethanol and ethyl acetate. Additionally, the n-hexane solvent extracts other compounds that also contribute significantly to antioxidant activity.

Higher polarity solvents, such as ethyl acetate and ethanol, tend to be more effective in extracting phenolic compounds compared to non-polar solvents, like n-hexane. Although there is a positive correlation between total phenol content and antioxidant activity, different results

indicate that antioxidant activity depends not only on the total amount of phenol but also on the type and structure of the extracted phenolic compounds between total phenol content and antioxidant activity may be due to the diversity of phenolic compounds and other bioactive compounds in the extract that contribute to antioxidant activity. Different solvents extract different compounds (Figure 3), each with varying antioxidant potential.

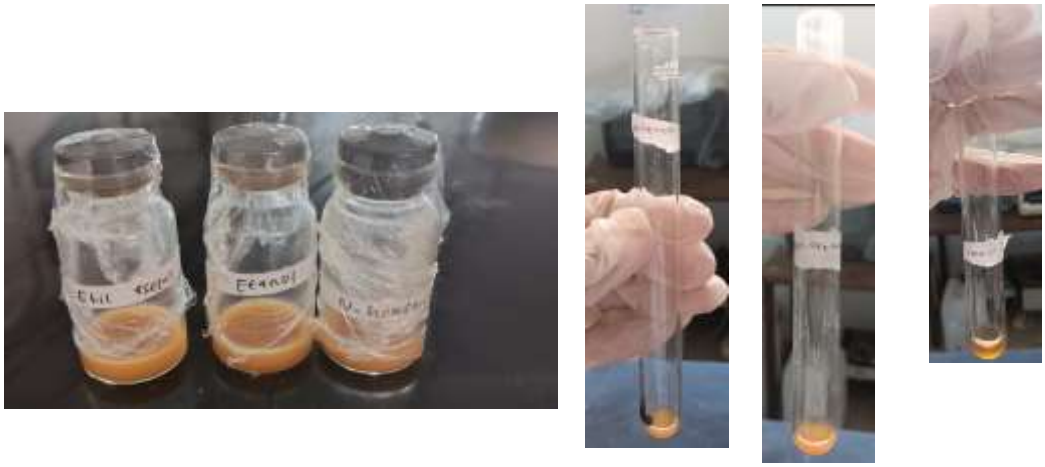

Figure 3. Image of turi stem extract with solvent fraction

Conclusion

The conclusion of this study shows that the type of solvent affects the total phenol content and antioxidant activity in turi leaf extract (*Sesbania grandiflora*). The highest total phenol content was obtained with ethyl acetate solvent (12.31 µg/mL), followed by ethanol (9 µg/mL), and the lowest in n-hexane (6.34 µg/mL). Ethyl acetate, a solvent with medium polarity, shows the best effectiveness in extracting phenolic compounds. However, antioxidant activity is not entirely in line with the total phenol content. The extract with n-hexane has the lowest IC50 (142.9 µg/mL), indicating the highest antioxidant activity, despite having a low phenol content. These results indicate that the type of phenolic compound or other compounds extracted influences antioxidant activity. This study emphasizes the importance of selecting the right solvent to maximize the content of bioactive compounds in accordance to the application's objectives.

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Conflicts of interest

The author declares that there is no conflict of interest in this research.

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